

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

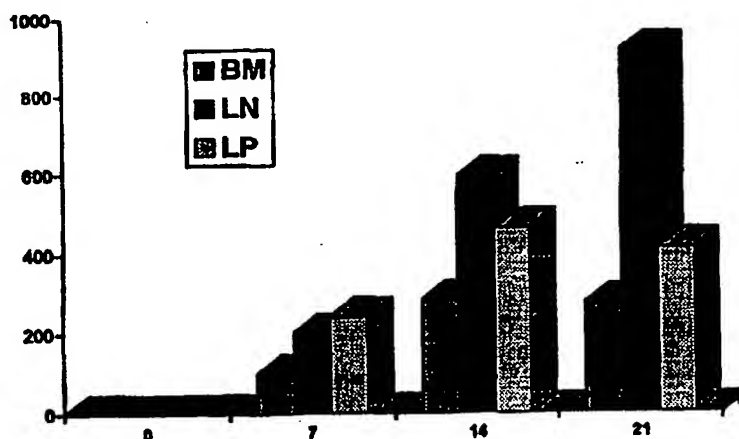
**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>A61K 38/20, 38/21, A61M 5/00</b>		<b>A2</b>	(11) International Publication Number: <b>WO 97/42968</b>
			(43) International Publication Date: 20 November 1997 (20.11.97)
(21) International Application Number: PCT/US97/08001 (22) International Filing Date: 12 May 1997 (12.05.97) (30) Priority Data: 08/649,121           14 May 1996 (14.05.96)      US 08/767,338           16 December 1996 (16.12.96)    US (71) Applicant: MAXIM PHARMACEUTICALS, INC. [US/US]; 3099 Science Park Road, San Diego, CA 92121 (US). (72) Inventors: HELLSTRAND, Kristoffer, Brödragatan 19, S-412 74 Göteborg (SE). HERMODSSON, Svante; Bergsbogatan 2, S-431 38 Mölndal (SE). (74) Agent: ALTMAN, Daniel, E.; Knobbe, Martens, Olson & Bear, 16th floor, 620 Newport Center Drive, Newport Beach, CA 92660 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  Published <i>Without international search report and to be republished          upon receipt of that report.</i>	

(54) Title: ADMINISTRATION OF HISTAMINE FOR THERAPEUTIC PURPOSES



## (57) Abstract

Methods for obtaining beneficial stable levels of circulating histamine are disclosed for use in methods for enhancing the cytotoxicity of cytotoxic effector cells. In such methods, a beneficial level of circulating histamine is attained and an agent whose ability to enhance natural killer cell cytotoxicity is augmented by histamine is administered. Alternatively, stable beneficial levels of circulating histamine can be attained in subjects receiving chemotherapy or antiviral treatment. The invention may also be employed in treatments combining histamine, agents which enhance the cytotoxicity of cytotoxic effector cells, and chemotherapeutic agents. Optimization of the delivery of histamine and substances which induce the release of endogenous histamine are also disclosed.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

## ADMINISTRATION OF HISTAMINE FOR THERAPEUTIC PURPOSES

### Background of the Invention

The present invention relates to methods of treating cancer or infectious disease in which histamine is administered in conjunction with additional agents. The additional agent may be an agent which stimulates the cytotoxic activity of natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) in a synergistic fashion with histamine. Alternatively, the additional agent may be a chemotherapeutic, antiviral, or antibiotic agent. Methods combining histamine, agents which act synergistically with histamine to increase the cytotoxicity of NK cells and CTLs, and chemotherapeutic agents are also contemplated.

The invention is based on the surprising observation that despite previous reports of histamine's short half life in the body, it is possible to attain stable beneficial levels of circulating blood histamine and to maintain these beneficial levels for hours or days after the last administration of histamine. This observation facilitates treatments in which histamine administration to obtain beneficial levels of circulating blood histamine is combined with treatment with other agents. The invention also relates to improvements in the method of administering histamine. A brief review of the observations leading to the present invention is provided below to place the present invention in context.

#### A. Cell Types Involved in the Generation of an Immune Response

Recent anticancer and antiviral strategies have focussed on utilizing the host immune system as a means of cancer or antiviral treatment or therapy. The immune system has evolved complex mechanisms for recognizing and destroying foreign cells or organisms present in the body of the host. Harnessing the body's immune mechanisms is an attractive approach to achieving effective treatment of malignancies and viral infections.

A wide array of effector cells, each having its own characteristics and role, implement the immune response. One type of effector cell, the B cell, generates antibodies targeted against foreign antigens encountered by the host. In combination with the complement system, antibodies direct the destruction of cells or organisms bearing the targeted antigen.

Another type of effector cell, the T cell, is divided into subcategories which play different roles in the immune response. Helper T cells secrete cytokines which stimulate the proliferation of other cells necessary for mounting an effective immune response, while suppressor T cells down regulate the immune response. A third category of T cell, the cytotoxic T cell (CTL), is capable of directly lysing a targeted cell presenting a foreign antigen on its surface.

An additional type of effector cell is the natural killer cell (NK cell), a type of lymphocyte having the capacity to spontaneously recognize and destroy a variety of malignant cell types. This characteristic of NK cells makes them an attractive candidate for exploitation in anticancer and antiviral treatments and therapies based on using the host's immune system as a weapon against malignant tumors and viruses.

#### B. Cytokines Involved Mediating the Immune Response

The interplay between the various effector cells listed above is influenced by the activities of a wide variety of chemical factors which serve to enhance or reduce the immune response as needed. Such chemical modulators may be produced by the effector cells themselves and may influence the activity of immune cells of the same or different type as the factor producing cell.

5 One category of chemical mediators of the immune response is cytokines, molecules which stimulate a proliferative response in the cellular components of the immune system.

Interleukin-2 (IL-2) is a cytokine synthesized by T cells which was first identified in conjunction with its role in the expansion of T cells in response to an antigen (Smith, K.A. Science 240:1169 (1988)). It is well known that IL-2 secretion is necessary for the full development of cytotoxic effector T cells (CTLs), which play an important  
10 role in the host defense against viruses. Several studies have also demonstrated that IL-2 has antitumor effects that make it an attractive agent for treating malignancies (see e.g. Lotze, M.T. et al, in "Interleukin 2", ed. K.A. Smith, Academic Press, Inc., San Diego, CA, p237 (1988); Rosenberg, S., Ann. Surgery 208:121 (1988)). In fact, IL-2 has been utilized to treat subjects suffering from malignant melanoma, renal cell carcinoma, and acute myelogenous leukemia. (Rosenberg, S.A. et al, N. Eng. J. Med. 316:889-897 (1987); Bukowski, R. M. et al., J. Clin. Oncol  
15 7:477-485 (1989); Foa, R. et al., Br. J. Haematol 77:491-496 (1990)).

It appears likely that NK cells are responsible for the anti-tumor effects of IL-2. For example, IL-2 rapidly and effectively augments the cytotoxicity of isolated human NK cells *in vitro* (Dempsey, R.A., et al., J. Immunol. 129:2504 (1982); Phillips, J.H., et al. J. Exp. Med. 170:291 (1989)). Thus, the cytotoxic activity of NK cells treated with IL-2 is greater than the constitutive levels of cytotoxicity observed in untreated cells. Furthermore, depletion  
20 of NK-cells from animals eliminates IL-2's antitumor effects. (Mule, J.J. et al, J. Immunol. Invest. 139:285 (1987); Lotze, M.T. et al., supra). Additional evidence for the role of NK cells results from the observation that NK cells are the only resting human peripheral blood lymphocytes expressing the IL-2 receptor on their cell surface. (Caligiuri, M.A. et al., J. Clin. Invest. 91:123-132 (1993)).

Another cytokine with promise as an anti-cancer and antiviral agent is interferon- $\alpha$ . Interferon- $\alpha$  (IFN- $\alpha$ )  
25 has been employed to treat leukemia, myeloma, and renal cell carcinomas. Isolated NK cells exhibit enhanced cytotoxicity in the presence of IFN- $\alpha$ . Thus, like IL-2, IFN- $\alpha$  also acts to augment NK cell mediated cytotoxicity. (Trinchieri, G. Adv. Immunol. 47:187-376 (1989)).

#### C. In vivo results of histamine and histamine agonist treatments

Histamine is a biogenic amine, i.e. an amino acid that possesses biological activity mediated by  
30 pharmacological receptors after decarboxylation. The role of histamine in immediate type hypersensitivity is well established. (Plaut, M. and Lichtenstein, L.M. 1982 Histamine and immune responses. In Pharmacology of Histamine Receptors, Ganellin, C.R. and M.E. Parsons eds. John Wright & Sons, Bristol pp. 392-435.)

Examinations of whether histamine or histamine antagonists can be applied to the treatment of cancer have yielded contradictory results. Some reports suggest that administration of histamine alone suppressed tumor growth  
35 in hosts having a malignancy. (Burtin, Cancer Lett. 12:195 (1981)). On the other hand, histamine has been reported to accelerate tumor growth in rodents (Nordlund, J.J. et al., J. Invest. Dermatol 81:28 (1983)).

Similarly, contradictory results were obtained when the effects of histamine receptor antagonists were evaluated. Some studies report that histamine receptor antagonists suppress tumor development in rodents and humans (Osband, M.E. et al., Lancet 1 (8221):636 (1981)). Other studies report that such treatment enhances tumor growth and may even induce tumors (Barna, B.P. et al., Oncology 40:43 (1983)).

#### 5 D. Synergistic Effects of Histamine and IL-2

Despite the conflicting results when histamine is administered alone, recent reports clearly reveal that histamine acts synergistically with cytokines to augment the cytotoxicity of NK cells and CTLs. Thus, therapies employing the combination of histamine and cytokines represent an attractive approach to anti-cancer strategies based on using the host immune system to attack the malignancy. Similarly, antiviral treatments using any of the  
10 well known antiviral agents is also contemplated.

Studies using histamine analogues suggest that histamine's synergistic effects are exerted through the H<sub>2</sub>-receptors expressed on the cell surface of monocytes. For example, the H<sub>2</sub>-receptor agonist dimaprit was capable of augmenting NK cell mediated cytotoxicity, while close structural analogues lacking biological activity failed to produce an effect. Additionally, H<sub>2</sub>-receptor antagonists blocked the effects of histamine and dimaprit, implicating  
15 the H<sub>2</sub>-receptor in the transduction of the histamine response. (Hellstrand, K. et al., J. Immunol. 137:656 (1986)).

Histamine's synergistic effect when combined with cytokines is not the result of a direct positive effect of histamine on NK cell and CTL cytotoxicity. Rather, the synergistic effects result from the suppression of a down regulation of cytotoxicity mediated by other cell types present along with the cytotoxic cells. The discussion below provides some of the evidence suggesting that histamine's synergistic effects result from the suppression of negative  
20 signals exerted by other cell types.

U.S. Patent Number 5,348,739 discloses the synergistic effects of histamine and interleukin-2. As discussed above, IL-2 normally induces a cytotoxic response in NK cells. *In vitro* studies with NK cells alone confirm that cytotoxicity is stimulated when IL-2 is administered. However, in the presence of monocytes, the IL-2 induced enhancement of cytotoxicity of NK cells is suppressed.

25 In the absence of monocytes, histamine had no effect or weakly suppressed NK mediated cytotoxicity. (U.S. Patent Number 5,348,739; Hellstrand, K. et al., J. Immunol. 137:656 (1986); Hellstrand, K. and Hermodsson, S., Int. Arch. Allergy Appl. Immunol. 92:379-389 (1990)). However, NK cells exposed to histamine and IL-2 in the presence of monocytes exhibit elevated levels of cytotoxicity relative to that obtained when NK cells are exposed only to IL-2 in the presence of monocytes. *Id.* Thus, the synergistic enhancement of NK cell cytotoxicity by combined histamine  
30 and interleukin-2 treatment results not from the direct action of histamine on NK cells but rather from suppression of an inhibitory signal generated by monocytes.

Without being limited to a particular mechanism, it is believed that the inhibitory effects of monocytes on cytotoxic effector cells such as NK cells and CTLs result from the generation of H<sub>2</sub>O<sub>2</sub> by monocytes. It has been reported that the production of H<sub>2</sub>O<sub>2</sub> by monocytes suppresses NK cell cytotoxicity. (Van Kessel, K.P.M. et al.,  
35 Immunology, 58:291-296 (1986); El-Hag, A. and Clark, R.A. J. Immunol. 133:3291-3297 (1984); Seaman, W.E. et al., J. Clin. Invest. 69:876-888(1982)). Further evidence of the role of H<sub>2</sub>O<sub>2</sub> in suppressing NK cell cytotoxicity comes

from *in vitro* studies showing that the addition of catalase, an enzyme which acts to remove  $H_2O_2$ , to preparations of monocytes and NK cells exposed to IL-2 removes the inhibitory effects of the monocytes. (Seaman, supra.) Thus, histamine may exert its synergistic effects by reducing the level of  $H_2O_2$  produced by monocytes. Hellstrand, K., Asea, A., Hermodsson, S. Histaminergic regulation of antibody-dependent cellular cytotoxicity of granulocytes, 5 monocytes and natural killer cells, J. Leukoc. Biol. 55:392-397 (1994).

Monocytes are not the only cell type which negatively regulates NK cell and CTL cytotoxicity. Experiments have demonstrated that granulocytes suppress both the constitutive and IL-2 induced cytotoxic activity of NK cells in vitro. Like the monocyte mediated suppression discussed above, granulocyte mediated suppression is synergistically overcome by treatment with IL-2 and histamine. (U.S. Patent Number 5,348,739; Hellstrand, K., Asea, A., 10 Hermodsson, S. Histaminergic regulation of antibody-dependent cellular cytotoxicity of granulocytes, monocytes and natural killer cells, J. Leukoc. Biol. 55:392-397 (1994)).

It appears that the  $H_2$ -receptor is involved in transducing histamine's synergistic effects on overcoming granulocyte mediated suppression. For example, the effect of histamine on granulocyte mediated suppression of antibody dependent cytotoxicity of NK cells was blocked by the  $H_2$ -receptor antagonist ranitidine and mimicked by 15 the  $H_2$  receptor agonist dimaprit. In contrast to the complete or nearly complete abrogation of monocyte mediated NK cell suppression by histamine and IL-2, such treatment only partially removed granulocyte mediated NK cell suppression. (U.S. Patent Number 5,348,739; Hellstrand, K. et al., Histaminergic regulation of antibody dependent cellular cytotoxicity of granulocytes, monocytes and natural killer cells., J. Leukoc. Biol. 55:392-397 (1994)).

As suggested by the experiments above, therapies employing histamine and cytokines are effective anti- 20 cancer strategies. U.S. Patent Number 5,348,739 discloses that mice given histamine and IL-2 prior to inoculation with melanoma cell lines were protected against the development of lung metastatic foci. This effect was a consequence of synergistic interaction between histamine and IL-2, as demonstrated by the significant reduction in metastatic foci observed in mice given histamine and IL-2 as compared to mice given histamine or IL-2 alone.

In addition to the synergistic effects observed in the assay of lung metastatic foci, synergistic effects of 25 histamine plus IL-2 treatment were also observed in assays in which NK cell cytotoxicity was measured by determining the ability of mice to kill malignant cell lines derived from both humans and mice which were injected into them. *Id.*

In studies conducted to investigate the role of histamine in NK-cell dependent protection against herpes simplex virus (HSV) type 2, it was discovered that a single dose of histamine could prolong survival time in animals 30 inoculated intravenously with HSV, and a synergistic effect on the survival time of animals treated with a combination of histamine and IL-2 was observed (Hellstrand, K. et al., Role of histamine in natural killer cell-dependent protection against herpes simplex virus type 2 infection in mice., Clin. Diagn. Lab. Immunol. 2:277-280 (1995)).

The above results demonstrate that strategies employing a combination of histamine and IL-2 are an 35 effective means of treating malignancies and viral infection.

#### E. Synergistic Effects of Histamine and Interferon- $\alpha$

Histamine also acts synergistically with interferon- $\alpha$  to overcome the suppression of NK cell cytotoxicity by monocytes (Hellstrand et al., Regulation of the NK cell response to interferon-alpha by biogenic amines, J. Interferon Res. 12:199-206 (1992)). Like IL-2, interferon- $\alpha$  augments NK cell constitutive NK cell cytotoxicity. *Id.* Monocytes suppress the interferon- $\alpha$  mediated enhancement of NK cell killing of malignant target cells *in vitro*.

5 Monocyte mediated suppression of NK cell cytotoxicity was overcome by treatment with histamine and interferon- $\alpha$ . The effects of histamine were blocked by H<sub>2</sub>-receptor antagonists and mimicked by H<sub>2</sub>-receptor agonists. Compounds bearing structural similarity to the H<sub>2</sub>-receptor agonist dimaprit but lacking the activity of the agonist were unable to act synergistically with interferon- $\alpha$ . (Hellstrand et al. J., Interferon Res. 12:199-206 (1992)).

F. Human Treatments Combining Histamine, Interleukin-2 and Interferon- $\alpha$

10 The *in vitro* and animal results discussed above suggested that histamine+IL-2+interferon- $\alpha$  was a promising method for treating human malignancies. In fact, combined histamine, IL-2, and interferon- $\alpha$  treatments have proven effective in the treatment of a variety of human malignancies, providing a 75% response rate significantly greater than that observed with IL-2 alone. (Hellstrand et al., Histamine in Immunotherapy of Advanced Melanoma: A Pilot Study, Cancer Immunology and Immunotherapy 39: 416-419 (1994)). In the above study, subjects received a  
15 constant infusion of IL-2 (Proleukin®, Eurocetus),  $18 \times 10^6$  U/m<sup>2</sup> on days 1-5 and 8-12, repeated every 4-8 weeks, as well as interferon- $\alpha$  ( $6 \times 10^6$  U daily, s.c.). In addition to the IL-2, eight of the subjects received histamine dihydrochloride (1 mg s.c.) twice daily. *Id.*

Only one subject in the group receiving IL-2 and interferon- $\alpha$  exhibited a partial or mixed response, a response rate of 14% (1 of 7). In contrast, the group receiving both IL-2, interferon- $\alpha$  and histamine showed an  
20 corresponding response rate of 75% (6 of 8). Only two of these subjects failed to respond. *Id.*

Thus, histamine+IL-2+interferon- $\alpha$  is an effective anti-cancer therapy.

G. Human Treatments with Histamine+

IL-2+Chemotherapeutic Agents

Recently, the efficacy of treatments employing histamine, IL-2, and chemotherapeutic agents was examined  
25 in humans suffering from acute myelogenous leukemia (AML). (Brune and Hellstrand, Remission Maintenance Therapy with Histamine and Interleukin-2 in Acute Myelogenous Leukemia, Br. J. Haematology, March 1996).

In one set of experiments, killing of AML blasts by NK cells was examined *in vitro*. IL-2 induced NK mediated cytotoxicity, but this effect was suppressed by monocytes. Histamine did not affect the IL-2 induced cytotoxic response in the absence of monocytes but blocked the suppressive effects of the monocytes. However,  
30 in the presence of the H<sub>2</sub>-receptor antagonist ranitidine, histamine was ineffective in overcoming the monocyte suppression. *Id.*

Additionally, AML patients in remission were treated with histamine+IL-2+the chemotherapeutic agents cytarabine and thioguanine and the duration of remission was measured and compared to the length of remissions prior to initiating the treatment. Five of the patients receiving histamine+IL-2+cytarabine+ thioguanine remained  
35 in complete remission ranging in duration from 9-27 months at the time of publication. Two patients relapsed after remissions lasting 8 and 33 months. In the five patients who had undergone a remission followed by a relapse prior



to initiation of the histamine+IL-2+cytarabine+thioguanine regimen, the duration of remissions following initiation of the regimen exceeded the duration of the prior remission. /d. Such "inversion of remission time" is rare in the natural course of AML and reportedly only occurs in a small fraction (< 10%) of AML patients treated with IL-2 as the single agent. Shepherd et al, Phase II Study of Subcutaneous rHU IL-2 in Patients with Acute Myelogenous  
5 Leukemia in Partial or Complete Second Remission and Partial Relapse, Br. J. Haematol. S87, 205 (1994).

Thus, histamine+IL-2+chemotherapeutic agents is an effective anti-cancer therapy.

#### H. Optimization of Histamine Delivery

Histamine is a strongly bioactive molecule with powerful biological effects. We have discovered that bolus doses of effective amounts of histamine have significant unwanted side effects, including flushing, discomfort,  
10 increased heart and respiratory rate, hypotension, and severe headache. At the same time, we have discovered that histamine-mediated therapy is most effective if provided in discrete dosages over a relatively short time period as opposed to infusion or controlled release over a period of days or weeks.

#### Summary of the Invention

The present invention relates to treatments combining the administration of histamine or histamine-inducing  
15 agents, to achieve a beneficial level of circulating blood histamine with treatment with a second agent which enhances cytotoxic effector cell cytotoxicity or is a chemotherapeutic agent. Combinations of histamine, agents which enhance NK cell cytotoxicity, and chemotherapeutic agents are also contemplated. It will be appreciated that the beneficial stable levels of circulating blood histamine may be attained by administering histamine before, during, after or throughout the course of the administration of the second agent. As used herein "stable" means a level  
20 that is maintained for hours or preferably, days.

Thus, the present invention provides for the use of histamine or a histamine-inducing compound in the preparation of a medicament for producing elevated, stable circulating levels of histamine in a patient. The medicament can be for administration in conjunction with a second therapeutic agent, such as a chemotherapeutic agent, and is useful for the treatment of a malignancy, a viral disease or a neoplastic disease.. The histamine or  
25 histamine-inducing compound is preferably selected from the group consisting of pharmaceutically acceptable forms of histamine, histamine dihydrochloride, histamine phosphate, histamine salts, histamine esters, histamine congeners, histamine prodrugs, H<sub>2</sub> receptor agonists, 5HT agonists, serotonin, retinoic acid, retinoids, IL-3 and ingestible allergens.

In accordance with another aspect of the invention, there is provided a composition comprising histamine  
30 or a histamine-inducing compound, together with a controlled release carrier. The carrier has the property of delivering the composition at a rate of about 0.025 to 0.2 mg/minute, at a rate which results in the release of about 0.025 to 0.2 mg/minute endogenous histamine, or for a period of time not less than one and not greater than thirty minutes. The controlled release carrier is preferably selected from the group consisting of polymers, gels, microspheres, liposomes, tablets, capsules, infusion pumps, syringe pumps, ocular inserts, transdermal formulations,  
35 hydrophobic gums, microcapsules, and colloidal drug delivery systems.

In accordance with yet another aspect of the present invention, there is provided a device for administering a therapeutically effective amount of a substance selected from the group consisting of pharmaceutically acceptable forms of histamine, histamine dihydrochloride, histamine phosphate, histamine salts, histamine esters, histamine congeners, histamine prodrugs, serotonin, 5HT agonists,  $H_2$  receptor agonists, substances which induces the release of an effective therapeutic amount of endogenous histamine, retinoic acid, retinoids, IL-3 and ingestible allergens. The device comprises an infusion device, a therapeutically effective amount of the substance, and a controller which delivers the substance over a period of time not less than one and not greater than thirty minutes. Preferably, the controller delivers the therapeutically effective amount of the substance over a period of time not less than five and not greater than twenty minutes. The therapeutically effective amount of the substance is about 0.4 to 10 mg/day, preferably about 2.0 mg/day, and is preferably delivered at a rate of about 0.025 to 0.2 mg/minute. The therapeutically effective amount of the substance can also result in the release of about 0.025 to 0.2 mg/minute endogenous histamine. The device preferably further comprises a controlled release carrier contained within the infusion device together with the substance.

In accordance with still another aspect of the present invention, there is provided a method for treating a patient having a malignancy, neoplastic disease or viral disease. The method comprises administering histamine or a histamine-inducing compound to the patient over a period of time such that a stable, elevated blood histamine concentration is achieved. Preferably, the method further comprises administering a second therapeutic agent, such as an anti-cancer or anti-viral agent. The histamine or histamine inducing compound and the second agent can be administered separately or together.

A more complete appreciation of the invention and many of the attendant advantages thereof will be readily perceived as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying figures.

Other objects, advantages and features of the present invention will become apparent to those skilled in the art from the following discussion.

#### Brief Description of the Drawings

Figure 1 shows that it is possible to obtain stable beneficial levels of circulating blood histamine.

#### Detailed Description of the Preferred Embodiment

Prior to the present invention, histamine was believed to have an extremely short half life, on the order of five minutes, in the blood. Beaven, M.A., Factors regulating availability of histamine at tissue receptors in Pharmacology of Histamine Receptors, C.R. Ganellin and M.E. Parsons eds., Wright PSG, Bristol, U.K. pp. 103-145 (1982). The present invention arose from the unexpected finding that, contrary to the prior reports, it is possible to achieve stable levels of circulating blood histamine lasting several hours or even days from the time of histamine administration. The present invention is the first report of stable circulating blood histamine. Using regimens involving the administration of both  $H_2$ -receptor antagonists and histamine, Burtin was able to obtain normal levels of circulating blood histamine in cancer subjects experiencing stabilization of the disease. (Burtin et al., Eur. J. Clin. Oncol. 24: 161-167 (1988)). However, the normal levels reported by Burtin were most likely a consequence of the

stabilization of the cancer rather than an observation of stable circulating levels of histamine for a significant period following administration. Evidence that the levels reported in Burtin were a consequence of physiological stabilization comes from the fact that the normal levels of histamine reported by Burtin when the subjects experienced a remission dropped to below normal levels before the subjects' deaths. Burtin did not specify the time after administration at which circulating histamine levels were measured, but it seems likely that the normal levels reported by Burtin were a consequence of endogenously produced histamine associated with stabilization of the cancer rather than an observation of persistence of extrinsically administered histamine.

Subjects suffering from cancer often exhibit decreased levels of circulating blood histamine. (Burtin et al. Decreased blood histamine levels in subjects with solid malignant tumors, Br. J. Cancer 47: 367-372 (1983)). Thus, the observation of stable and beneficial blood histamine levels lasting hours or days after histamine administration finds ready application to cancer and antiviral treatments based on synergistic effects between histamine and agents which enhance cytotoxic effector cell mediated cytotoxicity. In such protocols, the cytotoxic activity of NK cells and CTLs is enhanced by combining the administration of histamine to attain a stable level of circulating histamine sufficient to augment the activity of an agent which acts in synergy with histamine to increase cytotoxicity with the administration of the agent.

Additionally, chemotherapeutic treatments aimed at destroying the malignancy may result in lowered blood histamine levels. Example 1, below describes the decrease in blood histamine levels following treatment with the chemotherapeutic/cytostatic agents cytarabine and thioguanine.

#### EXAMPLE 1

In five patients with AML in remission, histamine levels in whole blood specimens were analyzed 1-5 days before and 1-2 weeks after treatment with cytarabine (16mg/m<sup>2</sup>/day subcutaneously) and thioguanine (40mg/day orally) for 21 days or until the platelet count was  $\leq 50 \times 10^9/L$ . Histamine was analyzed in heparinized venous blood using the radioimmunoassay available from Biomerica Inc., Newport Beach, CA 92663 (catalog no. 1051) according to the instructions which accompanied the assay kit.

In all patients, histamine levels declined after the treatment with cytostatics. (See Table below).

	Blood Histamine Before Patient Treatment ( $\mu$ moles/l)	Blood Histamine after Treatment ( $\mu$ moles/l)
1.	0.22	not detectable
2.	0.12	not detectable
3.	0.18	not detectable
4.	0.93	0.38
5.	0.24	0.14

The present invention may be used to restore or maintain beneficial stable blood histamine levels in subjects whose blood histamine levels have decreased as a consequence of chemotherapy.

Beneficial levels of circulating histamine can be achieved by administering histamine before, during, or after treatment with agents which enhance natural killer cell cytotoxicity or chemotherapeutic agents.

Thus, this invention relates to a method of augmenting the activity of an agent which enhances cytotoxic effector cell cytotoxicity comprising administering histamine such that a stable blood histamine concentration sufficient to augment the cytotoxicity enhancing effect of said agent is achieved, and administering a beneficial amount of said agent, wherein the cytotoxicity enhancing effects of said agent are augmented. As used herein, 5 "histamine" includes histamine, its dihydrochloride salt (histamine dihydrochloride), histamine phosphate, other histamine salts, esters, or prodrugs, and  $H_2$  receptor agonists. Serotonin and 5HT agonists are also contemplated. The administration of compounds which induce the release of endogenous histamine from the patient's own tissues are also included within the scope of the present invention; thus, the term "histamine" as used herein incorporates these compounds as well.

10 In one aspect of the present invention, the histamine is administered prior to administration of the agent which enhances NK cell and CTL cytotoxicity. In another embodiment, the histamine is administered following the administration of the agent which enhances cytotoxicity. In a further embodiment, the histamine is administered during the course of administration of the agent which enhances cytotoxicity. In another embodiment, the histamine is administered prior, during, and after the administration of the agent which enhances cytotoxicity.

15 In one embodiment, the histamine is administered at least 1 day prior to the administration of the agent which enhances cytotoxicity. In a preferred embodiment beneficial stable levels of circulating blood histamine are obtained by administering histamine at a dosage of 0.4 to 10 mg/day. In a further preferred embodiment, the histamine is administered over a period of 1 to 4 weeks. In a highly preferred embodiment, the histamine is administered for a period of 1-2 weeks. In one embodiment of the invention, the beneficial stable level of circulating 20 blood histamine concentration is at least  $0.2\mu\text{mol/L}$ .

In one embodiment of the present invention the cytotoxicity enhancing agent is at least one cytokine. Preferentially, the cytokine is interleukin-2. In a preferred aspect of the invention, the interleukin-2 is administered in an amount of  $0.5\text{--}50\mu\text{g/kg/day}$ . In a further preferred embodiment, the interleukin-2 is administered for a period of 1 day to 4 weeks.

25 In another embodiment of the invention, the cytokine is interferon- $\alpha$ . Preferentially, the interferon- $\alpha$  is administered at a dosage of 10,000-200,000 U/kg/day. For treatment of primary melanoma, the preferred dosage of interferon- $\alpha$  is 200,000 U/kg/day. For treatment of other cancers, the preferred dosage of interferon- $\alpha$  is 50,000 U/kg/day. In a further embodiment, the interferon- $\alpha$  is administered for a period of up to 18 months. Preferentially, the interferon- $\alpha$  is administered for a period of between 2-6 weeks.

30 In yet another embodiment, the cytotoxicity enhancing agent comprises interferon- $\alpha$  and interleukin-2.

Another aspect of the invention is a method of treating a subject having a malignancy with histamine and a second beneficial agent wherein the activity of said second beneficial agent is augmented by histamine comprising administering a pharmaceutically acceptable form of histamine such that a beneficial blood histamine level is achieved and administering said second beneficial agent.

In one embodiment of this aspect, the histamine is administered prior to the second beneficial agent. In a preferred embodiment of this aspect, the histamine is administered at least 1 day prior to the administration of the second beneficial agent.

In another embodiment of this invention, the histamine is administered after the second beneficial agent. In yet another embodiment, the histamine is administered during the course of administration of said second beneficial agent. In another aspect of the invention, the histamine is administered prior, during, and after the second beneficial agent.

Preferentially, the second beneficial agent acts to stimulate the cytotoxic activity of NK cells and CTLs.

A third aspect of the present invention is a method of enhancing the cytotoxic activity of NK cells and CTLs comprising

- a) measuring blood histamine levels in a subject to determine whether the cytotoxic activity of said subject's NK cells and CTLs could be enhanced by increasing the levels of blood histamine in said subject;
- b) administering a pharmaceutically acceptable form of histamine to a subject for whom said measuring step indicated that the cytotoxic activity of said subject's NK cells and CTLs could be enhanced by increasing said subject's blood histamine levels, such that beneficial levels of blood histamine are achieved; and
- c) administering a second agent to said subject having beneficial levels of blood histamine, such that the cytotoxic activity of said subject's NK cells and CTLs is enhanced.

In one aspect of the invention, the histamine is administered prior to the second agent. In a preferred version of this aspect, the histamine is administered at least 1 day prior to the administration of the second agent.

In another aspect of the invention, histamine is administered after the second agent. In another embodiment, histamine is administered during the administration of the second agent. In another embodiment, the histamine is administered prior, during, and after the administration of the second agent. In each instance, one embodiment of the invention includes an antiviral or antimicrobial agent as the second agent.

The present invention also includes a method of treating a malignancy comprising treating a subject having a malignancy with a chemotherapeutic agent and achieving and maintaining a beneficial stable level of circulating blood histamine by administering histamine in a dose sufficient to attain said beneficial stable levels of circulating blood histamine. Methods for administering chemotherapeutic agents are well established.

In one embodiment, the histamine is administered before the chemotherapeutic agent. In another embodiment, the histamine is administered after the chemotherapeutic agent. In another aspect of the invention, the histamine is administered during treatment with the chemotherapeutic agent. In yet another embodiment, the histamine is administered prior, during, and after treatment with the chemotherapeutic agent.

It will be appreciated that the subject's circulating blood histamine levels may be monitored during the course of treatment and boosted to beneficial levels whenever levels drop below beneficial levels or approach the lower limits of beneficial levels. For example, in this embodiment, histamine may be administered whenever the subject's histamine levels drop below 0.2  $\mu$ mole/L.

Alternatively, it will be appreciated that histamine may be administered at periodic intervals at dosages sufficient to establish and maintain beneficial levels.

Routes and carrier compositions for administering histamine and cytokines have been disclosed in U.S. Patent Number 5,348,739. Briefly, the histamine may be administered through a variety of routes, including 5 parenterally, intraperitoneally, subcutaneously, intravenously, intramuscularly, intraocularly, orally, or transdermally. Solutions of the active compound as a free acid or a pharmaceutically-acceptable salt may be administered in water with or without a surfactant such as hydroxypropyl cellulose. Dispersions are also contemplated such as those utilizing glycerol, liquid polyethylene glycols and mixtures thereof and oils. Antimicrobial compounds may also be added to the preparations. Injectable preparations may include sterile aqueous solutions or dispersions and powders 10 which may be diluted or suspended in a sterile environment prior to use. Carriers such as solvents dispersion media containing, e.g., water, ethanol polyols, vegetable oils and the like, may also be added. Coatings such as lecithin and surfactants may be utilized to maintain the proper fluidity of the composition. Isotonic agents such as sugars or sodium chloride may also be added as well as products intended for the delay of absorption of the active compounds such as aluminum monostearate and gelatin. Sterile injectable solutions are prepared as is known in the 15 art and filtered prior to storage and/or administration. Sterile powders may be vacuum dried freeze dried from a solution or suspension containing them.

Any material added to the pharmaceutical composition should be pharmaceutically-acceptable and substantially non-toxic in the amounts employed. Sustained-release preparations and formulations are also within the confines of this invention.

20 Pharmaceutically-acceptable carriers as utilized in the context of this patent include any and all solvents, dispersion media, coatings, antimicrobial agents, isotonic and absorption delaying agents and the like as is known in the art. All preparations are prepared in dosage unit forms for uniform dosage and ease of administration. Each dosage unit form contains a predetermined quantity of active ingredient calculated to produce a desired therapeutic effect in association with a required amount of pharmaceutical carrier. Methods for administering chemotherapeutic 25 agents are well established.

For the purposes of the above treatments, beneficial levels of blood histamine are at least 0.2 $\mu$ mole/L.

#### Stable Circulating Blood Histamine Levels

As described above, the present invention is based on the observation that it is possible to obtain beneficial and stable levels of circulating blood histamine. Example 2 depicts the stable beneficial levels of circulating blood 30 histamine obtained following histamine administration. Surprisingly, beneficial levels of circulating blood histamine persisted for considerable periods after histamine administration ceased.

#### **EXAMPLE 2**

Five patients with AML in remission received treatment with histamine dihydrochloride diluted in sterile 35 sodium chloride purchased from Apoteksbolaget, Umea, Sweden and human recombinant interleukin-2 (Proleukin®) obtained from the commercially available vial (Eurocetus, the Netherlands). Histamine and IL-2 were administered

morning and night at separate subcutaneous injection sites over a period of 21 consecutive days. The histamine was given as subcutaneous injections using 1 ml syringes containing 0.1 mg of histamine/ml. The histamine treatment was given twice daily (morning and night) at a dosage of 0.4-0.7mg histamine per injection (i.e. a daily total dose of histamine of 0.8-1.4 mg/day).

- 5 The IL-2 was given as subcutaneous injections using separate 1-ml syringes. The IL-2 syringes contained 50  $\mu$ g of IL-2/ml. The IL-2 treatment was given twice daily (morning and night), and the dose was 35-50  $\mu$ g of IL-2 per injection, i.e. a daily total dose of 70-100 $\mu$ g/day.

Peripheral blood venous samples were drawn in 10 ml heparinized test tubes before the onset of treatment and weekly thereafter. The samples were drawn at least 8 hours after the last injections of histamine and IL-2.

- 10 The concentration of histamine in the whole blood samples was analyzed by use of a double antibody radioimmunoassay kit obtained from Biomerica, Inc., Newport Beach, CA 92663 (catalog no. 1051). The manufacturer's instructions provided with the kit, dated June, 1989, were followed. Blood histamine levels were measured at the indicated times.

- Figure 1 shows the results of the experiments described above. Data are given as the concentration of  
15 histamine in micromoles/l (mean  $\pm$  standard error of the mean).

The subjects exhibited blood histamine levels of less than 0.2  $\mu$ mole/L at the start of the experiment. Following histamine administration, circulating blood histamine levels rose to beneficial levels. Surprisingly, the circulating blood histamine levels remained elevated for sustained periods of time, even after histamine administration was discontinued.

#### 20 Treatments Employing a Combination of Histamine and Interleukin-2

- The stable blood histamine levels discussed above find application in treatments in which NK cell and CTL cytotoxicity is augmented through the synergistic effects of histamine and agents which enhance cytotoxic effector cell cytotoxicity. As discussed above, one such enhancer of cytotoxicity is interleukin-2. Examples 3 and 4 describe methods of treatment in which a stable and beneficial level of histamine is achieved which augments the activity  
25 of IL-2.

#### EXAMPLE 3

- 0.5 mg/day of histamine in a pharmaceutically acceptable form is injected subcutaneously in a sterile carrier solution into subjects having a malignancy. One week later, after circulating blood histamine levels have increased to at least 0.2 $\mu$ mole/L, IL-2 administration is begun. Human recombinant IL-2 (Proleukin<sup>®</sup>, Eurocetus) is administered  
30 by continuous infusion of 27 $\mu$ g/kg/day on days 1-5 and 8-12.

The above procedure is repeated every 4-6 weeks until an objective regression of tumor disease is observed. The therapy may be continued even after a partial or complete response has been observed. In patients with complete responses, the therapy may be given with longer intervals between cycles.

- The treatment can additionally include monitoring circulating levels of blood histamine periodically and, when  
35 circulating histamine levels drop below 0.2 $\mu$ mole/L, injecting 0.5 mg/day of a pharmaceutically acceptable form of histamine to restore circulating blood histamine levels above 0.2 $\mu$ mole/L.

The treatment can also include periodically boosting circulating blood histamine levels by administering 0.5 mg/day histamine over a period of one to two weeks at regular intervals, such as bi-weekly, in order to maintain circulating blood histamine levels above 0.2  $\mu$ mole/L.

#### EXAMPLE 4

5 Human recombinant IL-2 (Proleukin<sup>®</sup>, Eurocetus) is administered by continuous infusion of 27  $\mu$ g/kg/day on days 1-5 and 8-12 into patients infected with herpes simplex virus (HSV) type 2. 0.5 mg/day of histamine in a pharmaceutically acceptable form is injected subcutaneously in a sterile carrier solution until circulating blood histamine levels have increased to at least 0.2  $\mu$ mole/L.

The above procedure is repeated every 4-6 weeks until an objective regression of the disease is observed.

10 The therapy may be continued even after a partial or complete response has been observed.

The treatment can additionally include monitoring circulating levels of blood histamine periodically and, when circulating histamine levels drop below 0.2  $\mu$ mole/L, injecting 0.5 mg/day of a pharmaceutically acceptable form of histamine to restore circulating blood histamine levels above 0.2  $\mu$ mole/L .

The treatment can also include periodically boosting circulating blood histamine levels by administering 0.5  
15 mg/day histamine over a period of one to two weeks at regular intervals, such as bi-weekly, in order to maintain circulating blood histamine levels above 0.2  $\mu$ mole/L.

If desired, 0.5 mg/day of histamine in a pharmaceutically acceptable form can also be injected subcutaneously for about one week prior to the start of treatment with IL-2, in order to increase circulating blood histamine levels to at least 0.2  $\mu$ mole/L.

#### 20 Combination of Histamine and Interferon- $\alpha$

Another enhancer of NK cell cytotoxicity is interferon- $\alpha$ . Example 5 describes methods of treatment in which a stable and beneficial level of histamine is achieved which augments the activity of interferon- $\alpha$ .

#### EXAMPLE 5

25 0.5 mg/day of histamine in a pharmaceutically acceptable form is injected subcutaneously in a sterile carrier solution into subjects having a malignancy or who have had a primary tumor surgically removed. One week later, after circulating blood histamine levels have increased to at least 0.2  $\mu$ mole/L, interferon- $\alpha$  administration is begun. Interferon is administered in doses of 50,000 U/kg/day in a suitable carrier solution for 2-6 weeks.

The above procedure is repeated 3 times a week, or even several times daily, for up to 24 months until  
30 an objective regression of the tumor is observed. The therapy with histamine, IL-2 and interferon may be continued even after a partial or complete response has been observed. In patients with complete responses, the therapy may be given with longer intervals between cycles.

The treatment can additionally include monitoring circulating levels of blood histamine periodically and, when circulating histamine levels drop below 0.2  $\mu$ mole/L, injecting an additional 0.5-2.0 mg/day of a pharmaceutically  
35 acceptable form of histamine to restore circulating blood histamine levels above 0.2  $\mu$ mole/L .



The treatment can also include periodically boosting circulating blood histamine levels by administering 0.5 mg/day histamine over a period of one to two weeks at regular intervals, such as bi-weekly, in order to maintain circulating blood histamine levels above 0.2 $\mu$ mole/L.

Additionally, the frequency of interferon- $\alpha$  administration may be varied depending on the patient's tolerance of the treatment and the success of the treatment. For example, interferon may be administered three times per week, or even daily, for a period of up to 24 months. Those skilled in the art are familiar varying interferon treatments to achieve both beneficial results and patient comfort.

Example 6 describes methods of treatment in which a stable and beneficial level of histamine is achieved which augments the activity of interferon- $\alpha$  in antiviral therapies.

10

#### EXAMPLE 6

0.5 mg/day of histamine in a pharmaceutically acceptable form is injected subcutaneously together with interferon- $\alpha$  in patients infected with hepatitis C. 0.5-2.0 mg/day histamine is administered in order to maintain circulating blood histamine levels above 0.2 $\mu$ mole/L. Interferon is administered in doses of 50,000 U/kg/day in a suitable carrier solution.

15

The above procedure is repeated 3 times a week, or even several times daily, for up to 24 months until normalized liver enzymes and clearance of viral RNA from serum is observed. The therapy with histamine and interferon may be continued even after a partial or complete response has been observed.

The treatment can additionally include monitoring circulating levels of blood histamine periodically and, when circulating histamine levels drop below 0.2 $\mu$ mole/L, injecting an additional 0.5-2.0 mg/day of a pharmaceutically acceptable form of histamine to restore circulating blood histamine levels above 0.2 $\mu$ mole/L.

20

The treatment can also include periodically boosting circulating blood histamine levels by administering 0.5-2.0 mg/day histamine over a period of one to two weeks at regular intervals, such as bi-weekly, in order to maintain circulating blood histamine levels above 0.2 $\mu$ mole/L.

0.5 mg/day of histamine in a pharmaceutically acceptable form can also be injected subcutaneously for about one week prior to the start of treatment with interferon, in order to increase circulating blood histamine levels to at least 0.2 $\mu$ mole/L.

25

Additionally, the frequency of interferon- $\alpha$  administration may be varied depending on the patient's tolerance of the treatment and the success of the treatment. For example, interferon may be administered three times per week, or even daily, for a period of up to 24 months. Those skilled in the art are familiar varying interferon treatments to achieve both beneficial results and patient comfort.

30

#### Combination of Histamine, IL-2 and Interferon- $\alpha$

Beneficial stable levels of circulating blood histamine can also be employed in conjunction with treatments involving several enhancers of NK cell cytotoxicity. Example 7 describes how to administer such treatments.

#### EXAMPLE 7

Subjects having a malignancy or viral infection such as hepatitis B, hepatitis C, human immunodeficiency virus (HIV), human papilloma virus (HPV) or herpes simplex virus (HSV) type 1 or 2 are administered human

35

recombinant IL-2 (Proleukin®, Eurocetus) by continuous infusion of 27 µg/kg/day on days 1-5 and 8-12. Additionally, subjects also receive a daily dose of  $6 \times 10^6$  U interferon- $\alpha$  administered by subcutaneous injection, and 0.5-2.0 mg/day of a pharmaceutically acceptable form of histamine. Histamine is administered for a period of one to two weeks until circulating blood histamine levels reach above 0.2 µmole/L.

5 The above procedure is repeated every 4-6 weeks until an objective regression of the tumor is observed, or until improvement in the viral infection occurs. The therapy may be continued even after a partial or complete response has been observed. In patients with complete responses, the therapy may be given with longer intervals between cycles.

The treatment can additionally include monitoring circulating levels of blood histamine periodically and, when  
10 circulating histamine levels drop below 0.2 µmole/L, injecting 0.5-2.0 mg/day of a pharmaceutically acceptable form of histamine for a period of one to two weeks to restore circulating blood histamine levels above 0.2 µmole/L.

The treatment can also include periodically boosting circulating blood histamine levels by administering histamine at regular intervals, such as daily, bi-weekly or weekly.

Histamine in a pharmaceutically acceptable form, such as a sterile carrier solution, can be injected  
15 subcutaneously 0.5-1.0 mg/injection, 1-4 time per day, for about one week prior to the start of treatment with IL-2 and interferon, in order to increase circulating blood histamine levels to at least 0.2 µmole/L.

Additionally, the frequency of interferon- $\alpha$  administration may be varied depending on the patient's tolerance of the treatment and the success of the treatment. For example, interferon may be administered three times per week, or even daily, for a period of up to 24 months. Those skilled in the art are familiar varying interferon  
20 treatments to achieve both beneficial results and patient comfort.

#### Combination of Histamine and Chemotherapeutic Agents

Histamine may also be used in conjunction with chemotherapeutic agents. Typically, levels of circulating histamine decline during chemotherapy. Low levels of circulating histamine may result in the suppression of NK cell cytotoxicity by monocytes. This monocyte mediated suppression may be eliminated by administration of histamine  
25 prior, during, following or throughout chemotherapy in order to return circulating histamine levels to normal.

Accordingly, the present invention contemplates the restoration of circulating blood histamine levels to normal levels in conjunction with chemotherapeutic agents. Additionally, the treatment may also include administration of IL-2 and/ or interferon- $\alpha$ .

Representative compounds used in cancer therapy include cyclophosphamide, chlorambucil, melphalan,  
30 estramustine, iphosphamide, prednimustin, busulphan, tiotepa, carmustin, lomustine, methotrexate, azathioprine, mercaptopurine, thioguanine, cytarabine, fluorouracil, vinblastine, vincristine, vindesine, etoposide, teniposide, dactinomycin, doxorubin, daunorubicin, epirubicin, bleomycin, nitomycin, cisplatin, carboplatin, procarbazine, amacrine, mitoxantron, tamoxifen, nilutamid, and aminoglutemide. Procedures for employing these compounds against malignancies are well established. In addition, other cancer therapy compounds may also be utilized in the present  
35 invention.

Malignancies against which the treatment may be directed include, but are not limited to, primary and metastatic malignant tumor disease, hematological malignancies such as acute and chronic myelogenous leukemia, acute and chronic lymphatic leukemia, multiple myeloma, Waldenstroms Macroglobulinemia, hairy cell leukemia, myelodysplastic syndrome, polycythaemia vera, and essential thrombocytosis.

5 As described above, histamine+IL-2 has proven an effective combination with traditional chemotherapeutic methods in treating acute myelogenous leukemia. (Brune and Hellstrand, Br. J. Haematology, March 1996). Procedures for using the present invention in combination with chemotherapeutic agents and IL-2 are presented in Example 8. It will be appreciated that beneficial stable levels of circulating histamine may also be employed in treatments using only chemotherapeutic agents.

10

#### EXAMPLE 8

Subjects with AML in first, second, subsequent or complete remission are treated in 21-day courses with IL-2 (35-50  $\mu$ g (equivalent to  $6.3 \cdot 9 \times 10^5$  IU) s.c. twice daily), repeated with six-week intermissions and continued until relapse. In cycle #1, patients receive three weeks of low dose chemotherapy consisting of 16mg/m<sup>2</sup>/day cytarabine, and 40 mg/day thioguanine. Thereafter, patients are injected subcutaneously with 0.5mg/day of a  
15 pharmaceutically acceptable form of histamine for a period of 1 week to boost circulating histamine to a stable beneficial level above 0.2 $\mu$ mole/L. Thereafter, patients receive 100 $\mu$ g of interleukin-2/day for three weeks. Circulating histamine levels are boosted to beneficial levels by administering 0.5mg/day of a pharmaceutically acceptable form of histamine during the second week of this period. Thereafter, the subjects are allowed to rest for one week.

20

After the rest period at the end of cycle 1, cycle #2 is initiated. 0.5 mg/injection twice a day of a pharmaceutically acceptable form of histamine in a sterile carrier solution is injected subcutaneously until circulating blood histamine levels of at least 0.2 $\mu$ mole/L are achieved. Cytarabine (16 mg/m<sup>2</sup>/day s.c.) and thioguanine (40 mg/day orally) are given for 21 days (or until the platelet count is  $\leq 50 \times 10^9/l$ ). In the middle week, patients receive 0.5mg/injection twice per day of a pharmaceutically acceptable form of histamine to boost circulating  
25 histamine to beneficial levels. At the end of the three week chemotherapy treatment, patients receive 0.5mg/injection twice per day of a pharmaceutically acceptable form of histamine for a week. Thereafter, patients receive 100 $\mu$ g/day of interleukin-2 for three weeks. Circulating histamine levels are boosted in the middle week of the three week IL-2 treatment as described above. Patients are permitted to rest for two weeks.

Thereafter, cycle #3 is initiated. Cycle #3 is identical to cycle #2.

30

Alternatively, circulating histamine levels may be periodically monitored throughout the above procedure and histamine may be administered whenever circulating levels drop below a desirable level in order to maintain a beneficial level of blood histamine above 0.2 $\mu$ mole/L. Additionally, histamine may be administered at regular intervals during the treatment to maintain beneficial circulating levels. Another alternative is to provide histamine in a depot or controlled release form.

35

Controlled release vehicles are well known to those of skill in the pharmaceutical sciences. The technology and products in this art are variably referred to as controlled release, sustained release, prolonged action, depot, repository, delayed action, retarded release and timed release; the words "controlled release" as used herein is intended to incorporate each of the foregoing technologies.

5 Numerous controlled release vehicles are known, including biodegradable or bioerodable polymers such as polylactic acid, polyglycolic acid, and regenerated collagen. Known controlled release drug delivery devices include tablets, capsules, gels, microspheres, liposomes, ocular inserts, minipumps, and other infusion devices such as pumps and syringes. Implantable or injectable polymer matrices, and transdermal formulations, from which active ingredients are slowly released are also well known and can be used in the present invention.

10 Suitable infusion devices for use in the present invention include syringe pumps, auto injector systems and minipumps. Exemplary devices include the Ambulatory Infusion Pump Drive, Model 30, available from Microject Corp., Salt Lake City, Utah, and the Baxa Syringe Infuser, available from Baxa Corporation, Englewood, Colorado. Any device capable of delivering histamine in the manner described below can be used in the methods of the present invention.

15 The infusion devices of the present invention preferably have an effective amount of histamine, histamine dihydrochloride, histamine phosphate, serotonin, a 5HT agonist, an  $H_2$  receptor agonist or a substance which induces the release of an effective therapeutic amount of endogenous histamine contained therein. The device can be pre-loaded with the desired substance during manufacture, or the device can be filled with the substance just prior to use. Pre-filled infusion pumps and syringe pumps are well known to those of skill in the art. The active substance  
20 can be part of a formulation which includes a controlled release carrier, if desired. A controller is used with the device to control the rate of administration and the amount of substance to be administered. The controller can be integral with the device or it can be a separate entity. It can be pre-set during manufacture, or set by the user just prior to use. Such controllers and their use with infusion devices are well known to those of skill in the art.

Controlled release oral formulations are also well known. Active compound is incorporated into a soluble  
25 or erodible matrix. Hydrophilic gums, such as hydroxymethylcellulose, are commonly used. A lubricating agent such as magnesium stearate, stearic acid, or calcium stearate can be used to aid in the tableting process.

For the purpose of parenteral administration, histamine or compounds which induce endogenous histamine release can be combined with distilled water, preferably buffered to an appropriate pH and having appropriate (e.g., isotonic) salt concentrations. Histamine formulations can be provided as a liquid or as a powder which is  
30 reconstituted before use. They can be provided as prepackaged vials, syringes, or injector systems.

Controlled release preparations can be achieved by the use of polymers to complex or absorb the histamine. The controlled delivery can be exercised by selecting appropriate macromolecule such as polyesters, polyamino acids, polyvinylpyrrolidone, ethylenevinyl acetate, methylcellulose, carboxymethylcellulose, and protamine sulfate, and the concentration of these macromolecule as well as the methods of incorporation are selected in order to control release  
35 of active compound.

Hydrogels, wherein the histamine compound is dissolved in an aqueous constituent to gradually release over time, can be prepared by copolymerization of hydrophilic mono-olefinic monomers such as ethylene glycol methacrylate. Matrix devices, wherein the histamine is dispersed in a matrix of carrier material, can be used. The carrier can be porous, non-porous, solid, semi-solid, permeable or impermeable. Alternatively, a device comprising a central reservoir of histamine surrounded by a rate controlling membrane can be used to control the release of histamine. Rate controlling membranes include ethylene-vinyl acetate copolymer or butylene terephthalate/polytetramethylene ether terephthalate. Use of silicon rubber depots are also contemplated.

Transdermal patches, steady state reservoirs sandwiched between an impervious backing and a membrane face, and transdermal formulations, can also be used. A rate-controlling outer microporous membrane, or micropockets of histamine dispersed throughout a silicone polymer matrix can be used to control the release rate. These transdermal patches and formulations can be used with or without use of a penetration enhancer such as dimethylsulfoxide (DMSO), combinations of sucrose fatty acid esters with a sulfoxide or phosphoric oxide, eugenol or Azone.

Another possible method to control the release of histamine is to incorporate the histamine into particles of a polymeric material such as polyesters, polyamino acids, hydrogels, poly lactic acid, or ethylene vinylacetate copolymers.

Alternatively, instead of incorporating histamine into these polymeric particles, it is possible to entrap the histamine in microcapsules prepared, for example, by coacervation techniques, or by interfacial polymerization, for example hydroxymethylcellulose or gelatin-microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules, or in macroemulsions. Such technology is well known to those of ordinary skill in pharmaceutical sciences.

Preferably, the histamine is injected, infused, or released into the patient at a rate of from about 0.025 to 0.2 mg/min. A rate of about 0.1 mg/min is preferred. The histamine is preferably administered over a period of time ranging from about 1, 3 or 5 minutes to about 30 minutes, with an upper limit of about 20 minutes being preferred, such that the total daily adult dose of histamine ranges from between about 0.4 to about 10.0 mg, with about 0.5 to about 2.0 mg being preferred. Histamine administered over longer periods of time, i.e., longer than about 30 minutes, has been found to result in decreased or lack of efficacy, while rapid administration over less than 1 - 3 minutes can cause more pronounced and serious side effects, which include anaphylaxis, heart failure, bronchospasm, pronounced flushing, discomfort, increased heart rate and respiratory rate, hypotension, and severe headache.

Administration of each dose of histamine can occur from once a day to up to about four times a day, with twice a day being preferred. Administration can be subcutaneous, intravenous, intramuscular, intraocular, oral, or transdermal, and can utilize direct hypodermic or other injection or infusion means, or can be mediated by a controlled release mechanism of the type disclosed above. Any controlled release vehicle or infusion device capable of administering a therapeutically effective amount of histamine over a period of time ranging from about 1 to about 30 minutes can be used.

In addition to histamine, histamine dihydrochloride, histamine phosphate, other histamine salts, esters, congeners, prodrugs, and  $H_2$  receptor agonists, the use of serotonin, 5HT agonists, and compounds which induce release of histamine from the patient's own tissues is also included within the scope of the present invention. Retinoic acid, other retinoids such as 9-cis-retinoic acid and all-trans-retinoic acid, IL-3 and ingestible allergens are 5 compounds which are known to induce the release of endogenous histamine. These compounds can be administered to the patient by oral, intravenous, intramuscular, subcutaneous, and other approved routes. However, the rate of administration should result in a release of endogenous histamine in the range of from about 0.05-2.0 mg/min.

Administration of each dose of a compound which induces histamine release can occur from once per day to up to about four times a day, with twice per day being preferred. Administration can be subcutaneous, 10 intravenous, intramuscular, intraocular, oral, or transdermal, and can incorporate a controlled release mechanism of the type disclosed above. Any controlled release vehicle capable of administering a therapeutically effective amount of a compound which induces histamine release over a period of time ranging from about one to about thirty minutes can be used.

**WHAT IS CLAIMED IS:**

1. Use of histamine or a histamine-inducing compound in the preparation of a medicament for producing elevated, stable circulating levels of histamine in a patient.
2. Use according to Claim 1, wherein said medicament is further for administration in conjunction with  
5 a second therapeutic agent.
3. Use according to Claim 2, wherein the second therapeutic agent is a chemotherapeutic agent.
4. Use according to any of Claims 1-3, wherein said histamine or histamine-inducing compound is selected from the group consisting of pharmaceutically acceptable forms of histamine, histamine dihydrochloride, histamine phosphate, histamine salts, histamine esters, histamine congeners, histamine prodrugs, H<sub>2</sub> receptor agonists,  
10 5HT agonists, serotonin, retinoic acid, retinoids, IL-3 and ingestible allergens.
5. Use according to any of Claims 1-4, wherein said medicament is for treating a malignancy, a viral disease or a neoplastic disease.
6. A composition comprising histamine or a histamine-inducing compound, together with a controlled release carrier, said carrier having the property of delivering said composition at a rate of about 0.025 to 0.2  
15 mg/minute, at a rate which results in the release of about 0.025 to 0.2 mg/minute endogenous histamine, or for a period of time not less than one and not greater than thirty minutes.
7. The composition of Claim 6, wherein said controlled release carrier is selected from the group consisting of polymers, gels, microspheres, liposomes, tablets, capsules, infusion pumps, syringe pumps, ocular inserts, transdermal formulations, hydrophobic gums, microcapsules, and colloidal drug delivery systems.
- 20 8. A device for administering a therapeutically effective amount of a substance selected from the group consisting of pharmaceutically acceptable forms of histamine, histamine dihydrochloride, histamine phosphate, histamine salts, histamine esters, histamine congeners, histamine prodrugs, serotonin, 5HT agonists, H<sub>2</sub> receptor agonists, substances which induces the release of an effective therapeutic amount of endogenous histamine, retinoic acid, retinoids, IL-3 and ingestible allergens comprising:  
25 an infusion device;  
said therapeutically effective amount of said substance; and  
a controller which delivers said substance over a period of time not less than one and not greater than thirty minutes.
9. The device of Claim 8, wherein said controller delivers said therapeutically effective amount of  
30 said substance over a period of time not less than five and not greater than twenty minutes.
10. The device of Claim 8, wherein said therapeutically effective amount of said substance is about 0.4 to 10 mg/day.
11. The device of Claim 8, wherein said therapeutically effective amount of said substance is about 2.0 mg/day.
- 35 12. The device of Claim 8, wherein said therapeutically effective amount of said substance results in the release of about 0.025 to 0.2 mg/minute endogenous histamine.

13. The device of Claim 8, wherein said controller delivers said therapeutically effective amount of said substance at a rate of about 0.025 to 0.2 mg/minute.

14. The device of Claim 8, further comprising a controlled release carrier contained within said infusion device together with said substance.

5 15. A method for treating a patient having a malignancy, neoplastic disease or viral disease, comprising:

a) administering histamine or a histamine inducing compound to said patient over a period of time such that a stable, elevated blood histamine concentration is achieved.

16. The method of Claim 15, further comprising administering a second therapeutic agent.

10 17. The method of Claim 16, wherein said second agent is an anti-cancer or anti-viral agent.

18. The method of Claim 16, wherein said histamine or histamine inducing compound and said second agent are administered separately.



1/1

